

Clinical microbiology

## Evidence-based review of probiotics for antibiotic-associated diarrhea and *Clostridium difficile* infections<sup>☆</sup>

Lynne V. McFarland<sup>a,b,\*</sup><sup>a</sup> Department of Health Services Research and Development, Puget Sound Veterans Administration Healthcare System, Seattle, WA, USA<sup>b</sup> Department of Medicinal Chemistry, Box 357610, School of Pharmacy, University of Washington, Seattle, WA, USA

## ARTICLE INFO

## Article history:

Received 27 August 2008

Received in revised form

12 August 2009

Accepted 1 September 2009

Available online 13 October 2009

## Keywords:

Probiotics

Lactobacillus

Saccharomyces

Yogurt

Antibiotic-associated diarrhea

*Clostridium difficile*

## ABSTRACT

Probiotics are living microbes taken to confer a health benefit on the host. Although probiotics have a long history of use in Europe and Asia and have been on the U.S. market for over 14 years, there is still confusion about how to effectively use them. The use of probiotics for the prevention of antibiotic-associated diarrhea (AAD) and the treatment of *Clostridium difficile* infections (CDI) has been tested in randomized controlled clinical trials.

This paper will review the evidence supporting probiotic therapy for these two diseases and also review the advantages and disadvantages of probiotics. The advantages of probiotic therapy include multiple mechanisms of action against pathogens, the ability to interact with the host's natural defense systems, survival to the target organ and a good risk to benefit ratio. Disadvantages of probiotics include lack of standardization for clinical trial designs, variations in regulatory standards, poor quality control for some products and infrequent serious adverse reactions. Overall, probiotics offer a promising strategy for the prevention and treatment for AAD and CDI

Published by Elsevier Ltd.

## 1. Introduction

Antibiotic associated diarrhea (AAD) is a common complication of most types of antibiotics, especially for broad-spectrum antibiotics such as clindamycin, beta-lactams and 3rd generation cephalosporins. Rates of AAD vary from 5 to 39% depending upon the type of antibiotic, age, health status of the host and type of environment (hospitalization, extended care facilities, etc.) [1,2]. AAD has been reported in a wide variety of populations including outpatients, hospitalized patients and residents of long-term care facilities [1]. The clinical presentation of AAD may be mild (uncomplicated diarrhea) or more severe (colitis), or result in toxic megacolon or death [3]. Consequences of AAD may result in extended hospital stays, increased medical care costs and increased diagnostic procedures [3,4]. Prevention of AAD has rested on discontinuing the inciting antibiotic or switching to an antibiotic with

a narrower spectrum of action, but there are no other current effective preventive measures for AAD.

*Clostridium difficile* infections (CDI) continues to persist as a leading cause of nosocomial gastrointestinal illness [5–7]. The rates of CDI have been increasing globally over the years. In the U.S., CDI rates have doubled from 2001–2005 to 301,200 cases in 2005 [8]. In the U.S., CDI rates are steadily increasing [9] and by 2010, projections estimate 450,000–750,000 cases of CDI per year in the U.S. [9,10]. Outbreaks of an emergent strain, BI/NAP1/027, caused large outbreaks of severe CDI with high rates of mortality in Canada during 2003–2005 [11]. In one prospective study, the average length of stay for a hospitalization due to a CDI recurrence was 8.8 + 8.6 days (ranging from 3 to 26 days) [12]. Other studies have documented that CDI extends hospital stays for hospitalized patients from 4 to 36 days [13–16]. In a study of 1034 CDI cases in Massachusetts during 2000, the average cost ranged from \$10,212 to \$13,675/patient [17], projecting a national cost of CDI of \$3.2 billion/year. There are only two standard antibiotic treatments for CDI (vancomycin and metronidazole) and the response rate of metronidazole has been declining [18]. In addition, 20–60% of patients may develop recurrent episodes of CDI despite additional antibiotic treatment. Although other investigational antibiotics are under development, no new antibiotics are superior to the two standard antibiotics.

Probiotics are “live microorganisms, which when administered in adequate amounts, confer a health benefit on the host” [19].

<sup>☆</sup> Presented at Anaerobe 2008. The 9th Biennial Congress of the Anaerobe Society of the Americas. Long Beach CA. June 24–27, 2008 as “Point-Counterpoint: Probiotics- panacea or poppycock?”.

\* Corresponding author. Department of Health Services Research and Development, Puget Sound VA, S-152, 1100 Olive Way #1400, Seattle, WA 98101 Administration Healthcare System, Seattle, WA 98101, USA. Tel.: +1 206 277 1095; fax: +1 206 764 2935.

E-mail address: [lynne.mcfarland@va.gov](mailto:lynne.mcfarland@va.gov)

Probiotics are available as capsules of freeze-dried or lyophilized culture supernatants, dried powder of heat-dried culture supernatants, mixed in diary foods (such as yogurts, cheese, milks, or ice cream) or other foods (kefir, chocolate, wafers) [20–22]. In contrast, a prebiotic is “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health” [23]. Synergistic combinations of probiotics and prebiotics are called synbiotics [23].

The interest in probiotics as therapy has increased dramatically since 1999 and the frequency of peer-reviewed randomized clinical trials has kept pace with the global interest in this innovative method of therapy. Most trials have been in the field of acute pediatric diarrhea, but the frequency for antibiotic-associated trials has increased, while the frequency of *C. difficile* trials has been limited (Fig. 1).

## 2. Antibiotic-associated diarrhea

A wide variety of study designs have been used to test probiotics, which makes direct comparison of probiotic trials difficult. One example of a typical study design for prevention of antibiotic-associated diarrhea (AAD) is to enroll a patient population receiving antibiotics for infections and then to randomly assign probiotic or placebo treatment for the duration of the antibiotic. It is important to have sufficiently long follow-up times (usually 4–6 weeks post-antibiotic cessation) to capture delayed-onset AAD. Kotowsha et al. enrolled 269 children who were taking antibiotics for ear or respiratory infections and randomized them to either *Saccharomyces boulardii* (500 mg/d) or placebo for the duration of the antibiotic treatment [24]. Even though the follow-up time was short (two weeks post-antibiotic), the frequency of diarrhea in the probiotic group was significantly less (3.4%) compared to 17.3% in the placebo group. The yeast probiotic was well tolerated by the children. In contrast, Lewis et al. did not find a significant reduction in AAD in a study of 69 elderly patients randomized to *S. boulardii* or placebo for 14 days [25]. This failure may have been due to a flawed study design, in that patients were only followed while on antibiotics and no follow-up was done after antibiotics were discontinued. Several studies have shown that AAD may occur while on antibiotics, but AAD may also be delayed for up to two months

(in up to 38% of patients) after antibiotics are discontinued [1,26]. In a randomized controlled trial of a probiotic mixture given to prevent AAD, the rate of AAD was similar during antibiotic treatment (6.2% probiotic versus 8.1% control,  $P=0.74$ ), but cases of delayed-onset AAD post-antibiotic treatment were significantly fewer in the probiotic group (5.7%,  $P=0.003$ ) compared to the control group (27.5%) [27]. The study by Lewis et al. therefore, may have missed a significant number of AAD cases due to too short a follow-up period.

As the intestinal microflora is composed of many different strains of bacteria and fungal strains, another strategy for probiotic use is to repopulate the disturbed intestine with a mixture of probiotic strains. Unfortunately, the identities of the specific strains of normal microflora responsible for colonization resistance are unknown, so the choice of which strains to include in a probiotic mixture is uncertain. Several types of mixtures have been tested in similar study designs as those described above. One recent trial tested a fermented drink with a mixture of *Lactobacillus casei* DN-114001 ( $2 \times 10^{10}$ /d), *Streptococcus thermophilus* ( $2 \times 10^{10}$ /d) and *Lactobacillus bulgaricus* ( $2 \times 10^9$ /d) against a control milkshake [27]. The study treatment was given randomly to hospitalized adults over 50 years old on antibiotics for the duration of the antibiotic and an additional week. Patients were followed for an additional four weeks for the development of antibiotic-associated diarrhea (AAD). The patients given the probiotic mixture reported significantly less AAD (12.3%,  $P < .05$ ) than those given the control (33.9%). No adverse reactions were reported.

Meta-analysis has been used to pool studies of different probiotic strains to obtain a pooled estimate of the efficacy of probiotics for the prevention of AAD (Fig. 2). A note of caution, however, although most meta-analyses have concluded that probiotics are effective for preventing AAD [28,29], it is inappropriate to conclude that *all* probiotic strains are effective. The effectiveness of probiotics is strain specific and disease-specific, so that the probiotic strain must be linked to the disease. Most probiotic meta-analyses have focused on one type of disease indication with a variety of probiotic strains (for example, antibiotic-associated diarrhea [28–30]). When there are sufficient numbers of clinical trials, a meta-analysis limited to one disease indication and one type of probiotic strain may reduce the heterogeneity of studies [31]. As an alternative, some meta-analyses have done sensitivity

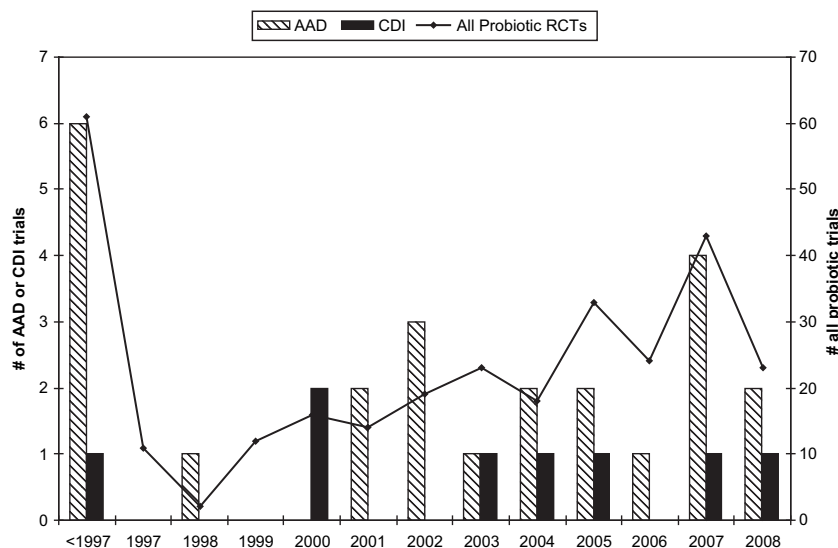
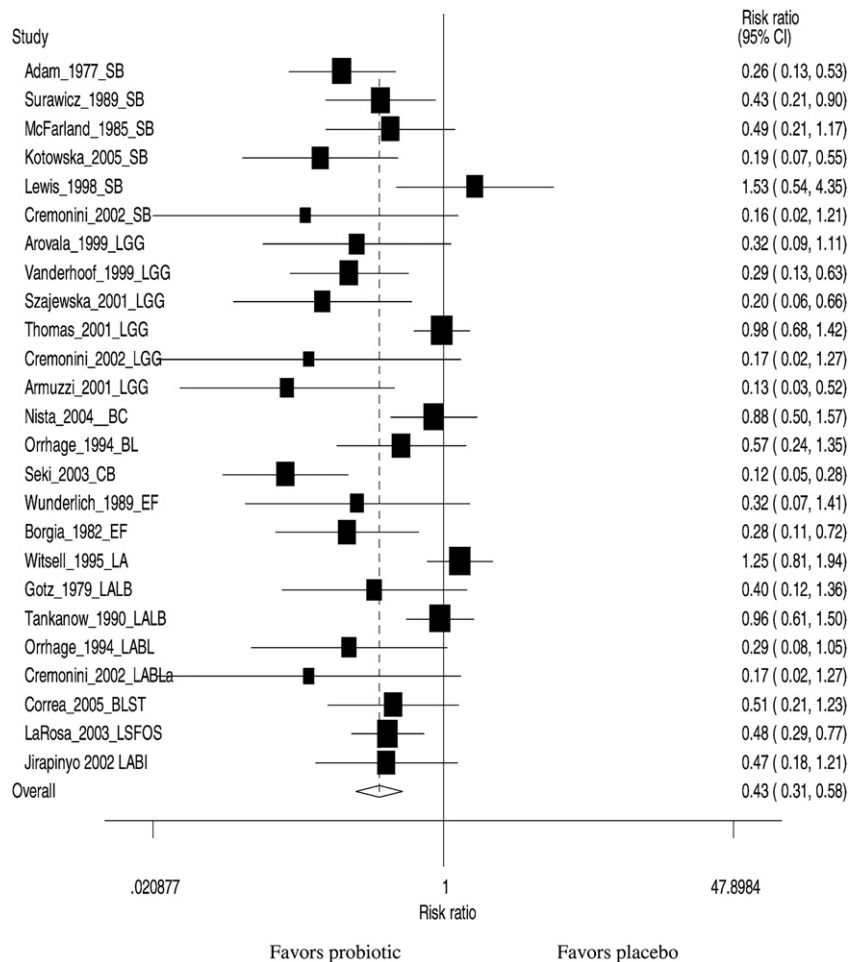


Fig. 1. Frequency of published peer-reviewed randomized controlled clinical trials in the scientific literature relating to probiotics over time (1985–2008), either all strains (line) or antibiotic-associated diarrhea (AAD) (hatched bars) or *Clostridium difficile* infections (CDI) (solid bars).



**Fig. 2.** Forest Plot of 25 randomized controlled trials of probiotics for the prevention of antibiotic-associated diarrhea showing crude and pooled risk ratios. SB = *Saccharomyces boulardii*; LGG = *Lactobacillus rhamnosus* GG; BC = *Bacillus clausii*; BL = *Bifidobacterium longum*; CB = *Clostridium butyricum* MIYAIRI; EF = *Enterococcus faecium* SF68; LA = *Lactobacillus acidophilus*; LALB = Lactinex = *L. acidophilus* and *L. bulgaricus*; LALB = *Lactobacillus acidophilus* and *Bifidobacterium longum*; LABLa = *Lactobacillus acidophilus* and *Bifidobacterium lactis*; BLST = *Bifidobacterium lactis* and *Streptococcus thermophilus*; LSFOS = *Lactobacillus sporogenes* and fructo-oligosaccharide; LABI = *Lactobacillus acidophilus* and *Bifidobacterium infantis*. Adapted from ref. [28].

analysis, separating out sub-groups by patient type (for example, just adults or just pediatrics) or by type of probiotic strain [28,32]. A sensitivity analysis found only two probiotic strains had sufficient evidence to show significant efficacy for the prevention of AAD, namely *S. boulardii* and *Lactobacillus rhamnosus* GG [28]. Although many meta-analyses have been done, pooling studies must be performed with these limitations in mind.

### 2.1. *C. difficile* disease

Probiotics may offer promise as an adjunctive therapy (given along with standard antibiotics vancomycin or metronidazole) for CDI, as several strains produce proteases that directly degrade *C. difficile* toxins or increase the immune response to *C. difficile* toxins A and B [33,34]. A meta-analysis of six randomized controlled trials using probiotics combined with one of the two standard antibiotics to treat CDI found probiotics, in general, significantly reduced the risk of *C. difficile* infections (combined RR = 0.59, 95% C.I. 0.41, 0.85,  $P = 0.005$ ) [28]. Although a variety of probiotic strains have been tested, most lack large randomized confirmatory placebo-controlled trials. In most cases, CDI was a secondary outcome for the trial and thus the trial was not powered to enroll sufficient numbers to detect a significant difference in CDI. A limited number of randomized controlled trials have been conducted to test probiotics

for the treatment of CDI as their primary outcome (Table 1) [35–39]. In one randomized, controlled trial, patients with CDI were prescribed either one of two doses of vancomycin (2 g/d or 500 mg/d) or metronidazole (1 g/d) then randomized to either *S. boulardii* or placebo (1 g/d for 4 weeks) [36]. Patients treated with the high dose vancomycin and the probiotic had significantly decreased recurrence rates (16.7%) compared to vancomycin and placebo (50%). The probiotic given with the low dose vancomycin or metronidazole was not significantly protective of CDI. This finding was in contrast to a prior trial of the same probiotic strain that showed significant effectiveness of *S. boulardii* as an adjunct to standard vancomycin or metronidazole therapy [35]. Several other trials for CDI were terminated early due to slow enrollment rates and the resulting small study sizes (15–25) precluded any statistical conclusions [37–39]. Although several clinical trials are underway currently testing other strains of probiotics for the treatment of CDI, no further studies have been published.

Some evidence may be inferred in studies that collect data on CDI as one of their secondary outcomes. In a study testing a probiotic mix for the prevention of antibiotic-associated diarrhea, a secondary outcome was the prevention of CDI [27]. Patients were randomized to either a probiotic mix of *L. casei*, *L. bulgaricus* and *Streptococcus thermophilus* (Actimel drink) at a dose of  $2.2 \times 10^8$  cfu/day or a placebo drink for duration of antibiotic plus

**Table 1**  
Randomized, controlled clinical trials of probiotics for the treatment of *Clostridium difficile* infections.

Probiotic	Population	Daily dose	Duration (days)	Frequency of CDI relapses in probiotic	Frequency of CDI relapses in controls	References
<i>S. boulardii</i>	124 adult patients on varied doses of vancomycin or metronidazole; recurrent and initial CDAD cases	$3 \times 10^{10}$	28, followed for another 4 weeks	15/57 (26.3%)	30/67 (44.8%)	McFarland 1994 [35]
<i>S. boulardii</i>	National, 1993–1996, 170 adult patients recurrent CDAD; on vancomycin (500 mg/d, n = 83) or (2 g/d, n=32) or metro (1 g/d, n = 53)	$2 \times 10^{10}$	28, followed for another 4 weeks	Vanco (2g/d) 3 (17%) Vanco (500 mg/d) 23 (51%) ns Metro (1g/d) 13 (48.1%) ns	7 (50%) 17 (44.7%) 13 (50%)	Surawicz 2000 [36]
<i>Lactobacillus rhamnosus</i> GG	25 Adults on vancomycin or metronidazole, recurrent and initial CDAD	Not reported	21	4/11 36.4% ns	5/14 35.7%	Pochapin 2000 [37]
<i>L. plantarum</i> 299v	29 Enrolled, 20 adults finished, 9 sites, 1–5 prior episodes, Over 2 yrs	$5 \times 10^{10}$	38 days, followed until Day 70	4/11 (36%) ns	Metro only, 6/9 (67%)	Wullt 2003 [38]
<i>L. rhamnosus</i> GG and 64 mg inulin	15 Adults on vanco or metro with CDAD, Enrolled over 9 months	$8 \times 10^{10}$	Duration of abx + 21 days	3/8 (37.5%) ns	1/7 (14.3%)	Lawrence 2005 [39]

\*  $P < 0.05$  compared to controls, ns=not significant, abx=antibiotics

one week. Of the 113 completing the trial, 0/56 (0%) developed CDI in the probiotic group compared to 9/53 (17%) in the placebo drink group,  $P < 0.05$ . The estimated cost of preventing one case of CDI with probiotic was \$120.00. Although CDI is an important disease, a limitation of the current probiotic research is a lack of trials of different types of probiotics.

Thusfar, there has only been one published trial using a probiotic mixture of two strains for the prevention of CDI, and there was no significance difference in the rate of CDI in the probiotic group (2.9%) compared with the control group (7.2%) [40]. Despite these limitations, several probiotics are currently under development for CDI including *Lactobacillus acidophilus*, *S. boulardii*, *Clostridium butyricum* and a non-toxicogenic strain of *C. difficile*.

### 3. Safety

The use of a living organism as therapy raises the potential for risk in several areas: transfer of antibiotic-resistance genes, translocation of the living organism from the intestine to other areas of the body, persistence in the intestines or the development of adverse reactions relating to interactions with the host's microflora. Fortunately, most of these concerns has not been a problem based on the decades of use in Europe and Asia. In Finland, where *L. rhamnosus* GG was been widely used as a probiotic since 1990, there has been no increase in reported adverse reactions or cases of *Lactobacillus* bacteremia attributable to probiotic strains [41]. In the clinical trials testing probiotics, no reports of bacteremia or fungemia have been associated with probiotic use. A review of the literature by the author (LVM) found 12 cases of *Lactobacillus* bacteremia in patients taking a *Lactobacillus* probiotic, mostly in children (9, 75%). There are also 24 cases of fungemia in patients

associated with the probiotic *S. boulardii*, mostly in severely ill children and adults [41]. *Bacillus subtilis* probiotic products had rare cases of bacteremia in 1988–1998, but no current cases have been reported.

Translocation from the intestines to other organs of the body is another potential risk of a living therapeutic. However, most of the probiotics that have been studied in animal models of translocation only show extra-intestinal presence of the probiotic if the animal has been compromised by high antibiotic exposure or is immunocompromised [42]. Only one case of a liver abscess in a 74-year-old severely ill woman was found and the strain isolated from the abscess was identical to the probiotic strain she had been ingesting for 4 months [43]. No cases of translocation have been reported in clinical trials of probiotics for AAD or CDI.

Most clinical trials of AAD and CDI have not reported any serious adverse reactions attributable to the probiotic treatment. The types of mild-moderate adverse reactions typically reported in probiotic trials include nausea, vomiting, abdominal cramps or pain, rash, diarrhea or constipation, but the frequencies are infrequently higher than the control group.

### 4. Advantages of probiotic therapy

Probiotics offer several advantages and have few disadvantages as a therapeutic mode for AAD and CDI (Table 2).

#### 4.1. Diverse mechanisms of action

A unique advantage of probiotic therapy is these living organisms incorporate a delivery system (most probiotics survive to the target organ) and bring an arsenal of anti-pathogenic strategies into

**Table 2**  
Advantages, disadvantages and recommendations for probiotics for the prevention of antibiotic-associated diarrhea and treatment of *Clostridium difficile* disease.

Advantages	Disadvantages	Future research needed
Multiple mechanisms of action possible	Strain specific effects	Standardized protocols
<i>In situ</i> delivery vehicle	Heterogeneous trials	Confirmatory studies on same strains
Safe in diverse patient populations	Risks in immunocompromised	Document risks/benefits
Diversity of potential organisms	Lack of quality control regulations	Cost/benefit analysis
Aids natural body defenses		Use of adequate dose ( $10^8$ – $10^{10}$ /day)
Lack of significant drug interactions		Expand types of strains tested
Ease of administration		
Inexpensive		

play. Potential mechanisms of action may include: (1) enhancing the natural barrier effect of normal intestinal microflora, (2) modulation of the immune system, (3) direct anti-microbial effects and (4) regulation of intestinal enzymes and interactions with the enteric nervous system [20,44–46]. Newer techniques, including metagenomics and PCR probes have documented that a typical human may carry over 40,000 bacterial species in the collective intestinal microbiome [47]. The normal intestinal flora has many functions, including digestion of food, but the one that is most germane for this discussion is called “colonization resistance” [48]. This involves the interaction of many bacterial microflora and results in a barrier effect against colonization of pathogenic organisms. Normal microflora may act by competitive exclusion of nutrients or attachment sites, produce bacteriocins, or produce enzymes detrimental to pathogenic growth. Factors that disrupt this protective barrier, for example antibiotic use or surgery, results in host susceptibility to pathogen colonization until such time as the normal microflora can become re-established. Probiotics are uniquely qualified to fit into this window of susceptibility and may act as surrogate normal microflora until recovery is achieved. There are several avenues to preserve the barrier effect: probiotics have been shown to protect the integrity of the tight junction between enterocytes [49], or block the attachment sites for pathogens (including *C. difficile*) or their toxins [33,50]. Some probiotics may directly destroy pathogenic toxins produced by *C. difficile* toxin A or B [51] or suspected etiologies for some cases of AAD (enterotoxigenic *E. coli*) [52,53]. Probiotics may also regulate immune responses, either by increasing secretory IgA levels in the intestines [33], by either increasing or deregulating cytokines [34] or inducing higher levels of anti-toxin A/B antibodies [54]. Probiotics may also alter amino acid metabolism, restoring protective levels of short-chain fatty acids in the intestine [55]. Probiotics also affect the regulation of the enteric nervous system [56] and reduce epithelial apoptosis [57]. Not all probiotics have the ability to produce every mechanism of action described above, but many of the strains utilize multiple mechanisms, increasing the probability of probiotic effectiveness against a specific pathogen. The benefit of these multiple mechanisms is the rapid restoration of bacteria disrupted by inciting antibiotics [58].

#### 4.2. Survival to target site

Another advantage of probiotics is that they can act as their own delivery vehicle for anti-pathogenic enzymes or defensive mechanisms. As all of the protective mechanisms described above are an inherent component of the probiotic organism and the enzymes are pre-packaged in a living organism, delivery of the multiple mechanisms of action are carried along when the organism passes through the digestive system. Pharmacokinetic studies in animal models or healthy human volunteers find that probiotic organisms survive passage and are detectable in the stool. Although much of the oral dose is destroyed (usually stool levels are 100 times lower than the oral dose given), the surviving dose is usually effective as a therapy as long as stool levels are over  $10^8$  organisms/g stool [59]. Few clinical trials using probiotics for AAD or CDI have documented the level of organisms present in the terminal site (colonic lumen). In one trial of patients with recurrent CDI given *S. boulardii* ( $2 \times 10^{10}$ /day) for 28 days, patients who had a subsequent CDI recurrence were found to have significantly lower numbers of *S. boulardii* ( $2 \times 10^4$ /gram stool) compared to those who did not recur ( $1 \times 10^6$ /g stool) [60].

### 5. Disadvantages/limitations

There are several issues relating to the field of probiotics including the wide diversity in both the types and quality of clinical

trials, the limited numbers of trials that have been properly conducted, the quality control of probiotic products on the market and the potential risks associated with probiotic use.

#### 5.1. Dose differences

There is still no consensus on the most effective dose of a probiotic. The range of daily doses in clinical trials has ranged from  $10^7$ /day to  $10^{11}$ /day [28]. Using an effective daily dose is an important consideration, as several meta-analyses have found efficacy at only the higher doses of probiotic. In 25 RCT trials of probiotics for the prevention of AAD, 8/12 (67%) of trials using a probiotic dose  $>10^{10}$ /day were significantly protective compared to only 2/12 (17%) if the dose was  $<10^{10}$ /day [28]. Unfortunately, many probiotics trials still fail to use sufficiently high daily doses of probiotics in their trials. Another issue is that a dose that is found to be effective for one probiotic strain may not be effective for another.

#### 5.2. Quality Control of probiotic products

A disadvantage to the dietary supplement regulations for probiotics is the lack of stringent quality control regulations for foods and dietary supplements. Some probiotic products are manufactured by well-established pharmaceutical companies with existing quality control protocols and batch standards. However, many probiotic products are not regulated and are manufactured in uncontrolled environments. The result is that some probiotic products contain the specific strains and concentrations stated on their labels and some do not. In a recent study where 14 U.S. commercial probiotic products were assayed, only 1/14 (7%) contained the bacteria listed on their label (most had other strains or contaminants) [61]. In another study of 18 commercial probiotic products, 39% had fewer organisms than stated on their label [62], which was similar to a study in the U.K. of 10 Bifidobacterial probiotic yogurts found  $10 \times 6$  fewer organisms than that listed on the labels [63]. In another large study of 58 probiotic products from Europe, U.K., Asia and Canada, they could not detect the probiotic on the label in 30% of the products and 60% had fewer organisms in the product than stated on the label ( $<10^6$ /g) [64]. In contrast, probiotic products manufactured by established pharmaceutical companies typically have the stated concentration and bacterial or fungal strain listed on the label.

### 6. Future research

Future research involves the development of newer technology for delivery mechanisms including nano-encapsulation, water-protected macrocapsules; recombinant strains responding to specific triggers in the host, and other microbiologic and biochemical developments [65]. Probiotics offer promise for a wide diversity of diseases and have an excellent safety-benefit ratio. However, the efficacy is disease and probiotic strain specific. Future research on different strains and mixtures, further understanding into the pathogenesis and mechanisms of action and safety are required.

Overall, probiotics are a promising strategy for the prevention of AAD. Although the pre-clinical and indirect clinical evidence is promising for CDI, more research is needed to find more probiotic strains that are effective adjuncts to vancomycin or metronidazole. More research is required using well done randomized controlled trials of sufficient size to detect significant differences.

### VA Disclaimer

The views expressed in this article are those of the author and do not represent the views of the Department of Veterans Affairs.

## References

- [1] McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. *Dig Dis* 1998;16:292–307.
- [2] Surawicz CM. Antibiotic-associated diarrhea and pseudomembranous colitis: are they less common with poorly absorbed antimicrobials? *Chemotherapy* 2005;51(Suppl. 1):81–9.
- [3] McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol.* 2008 Oct;3:563–78.
- [4] Dubberke ER, Reske KA, Olsen MA, McDonald LC, Fraser VJ. Short- and long-term attributable costs of *Clostridium difficile*-associated disease in nonsurgical inpatients. *Clin Infect Dis* 2008;46(4):497–504.
- [5] Song X, Bartlett JG, Speck K, Naegeli A, Carroll K, Perl TM. Rising Economic impact of *Clostridium difficile*-associated disease in adult hospitalized patient population. *Infect Control Hosp Epidemiol* 2008 Jul 18.
- [6] McFarland LV, Mulligan ME, Kwok RYY, Stamm WE. Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 1989;320:204–10.
- [7] Shek FW, Stacey BS, Rendell J, Hellier MD, Hanson PJ. The rise of *Clostridium difficile*: the effect of length of stay, patient age and antibiotic use. *J Hosp Infect* 2000 Jul;45(3):235–7.
- [8] Elixhauser A, Jhung M. *Clostridium difficile*-associated disease in U.S. Hospitals, 1993–2005. Statistical brief #50 Available at: Agency for Healthcare Research and Quality [www.hcup-us.ahrq.gov/reports/statbriefs/Sb50.pdf](http://www.hcup-us.ahrq.gov/reports/statbriefs/Sb50.pdf); April 2008.
- [9] Ziiberberg MD, Shorr AF, Kollef MH. Increase in adult *Clostridium difficile*-related hospitalizations and case-fatality rate, United States, 2000–2005. *Emerging Infect Dis* 2008 Jun;14(6):929–31.
- [10] McFarland LV, Clarridge JE, Beneda HW, Raugi GR. Fluoroquinolone use and risk factors for *Clostridium difficile* disease within a Veterans Administration Health Care System. *Clin Infect Dis* 2007;45(9):1141–51.
- [11] Pepin J, Valiquette L, Cossette B. Mortality attributable to nosocomial *Clostridium difficile*-associated disease during an epidemic caused by a hypervirulent strain in Quebec. *CMAJ* 2005;173(9):1037–42.
- [12] McFarland LV, Surawicz CM, Rubin M, Fekety R, Elmer GW, Greenberg RN. Recurrent *Clostridium difficile* disease: epidemiology and clinical characteristics. *Infect Control Hosp Epidemiol* 1999;20:43–50.
- [13] McFarland LV, Surawicz CM, Stamm WE. Risk factors for *Clostridium difficile* carriage and *C. difficile*-associated diarrhea in a cohort of hospitalized patients. *J Infect Dis* 1990;162(3):678–84.
- [14] Eriksson S, Aronsson B. Medical implications of nosocomial infection with *Clostridium difficile*. *Scand J Infect Dis* 1989;21(6):733–4.
- [15] Al Eidan FA, McElnay JC, Scott MG, Kearney MP. *Clostridium difficile*-associated diarrhoea in hospitalised patients. *J Clin Pharm Ther* 2000;25(2):101–9.
- [16] Kyne L, Hamel MB, Polavaram R, Kelly CP. Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clin Infect Dis* 2002 Feb 1;34(3):346–53.
- [17] O'Brien JA, Lahue BJ, Caro JJ, Davidson DM. The emerging infectious challenge of *Clostridium difficile*-associated disease in Massachusetts hospitals: clinical and economic consequences. *Infect Control Hosp Epidemiol* 2007 Nov;28(11):1219–27.
- [18] McFarland LV, Beneda HW, Clarridge JE, Raugi GJ. Implications of the changing face of *C. difficile* disease for health care practitioners. *Am J Infect Control* 2007;35(4):237–53.
- [19] Hoffman FA, Heimbach JT, Sanders ME, Hibberd PL. Executive summary: scientific and regulatory challenges of development of probiotics as foods and drugs. *Clin Infect Dis* 2008 Feb 1;46(Suppl. 2):S53–7. PMID: 18181723.
- [20] Elmer GW, McFarland LV, McFarland M. The power of probiotics: improving your health with beneficial microbes [Chapter 1 Introduction, pages]. Binghamton, N.Y.: Haworth Press; 2007. 1–24.
- [21] Homayouni A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chem* 2008;111(1):50–5.
- [22] Anukam KC, Osazuwa EO, Osadolor HB, Bruce AV, Reid G. Yogurt containing probiotic *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 helps resolve moderate diarrhea and increases CD4 count in HIV/AIDS patients. *J Clin Gastroenterol* 2008 Mar;42(3):239–43.
- [23] De Vrese M, Schrezenmeier J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol* 2008;111:1–66.
- [24] Kotowska M, Albrecht P, Szajewska H. *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Aliment Pharmacol Ther* 2005;21:583–90.
- [25] Lewis SJ, Potts LF, Barry RE. The lack of therapeutic effect of *Saccharomyces boulardii* in the prevention of antibiotic-related diarrhoea in elderly patients. *J Infect* 1998;36:171–4.
- [26] McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, et al. Prevention of  $\beta$ -lactam-associated diarrhea by *Saccharomyces boulardii* compared to placebo. *Am J Gastroenterol* 1995;90:439–48.
- [27] Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, et al. Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* 2007 Jul 14;335(7610):80–3.
- [28] McFarland LV. Meta-analysis of probiotics for prevention of antibiotic associated diarrhea and treatment of *Clostridium difficile* disease. *Am J Gastroenterol* 2006;101:812–22.
- [29] Meerpohl JJ, Timmer A. News from the Cochrane Library: probiotics for the prevention of paediatric antibiotic-associated diarrhoea. *Z Gastroenterol* 2007 Aug;45(8):715–7 [PMID: 17701862].
- [30] Johnston BC, Supina AL, Ospina M, Vohra S. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2007 Apr 18;(2):CD004827.
- [31] Van Niel CW, Feudtner C, Garrison MM, Christakis DA. *Lactobacillus* therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002;109:678–84.
- [32] Johnston BC, Supina AL, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. *CMAJ* 2006 Aug 15;175(4):377–83.
- [33] Buts JP. Twenty-five years of research on *Saccharomyces boulardii* trophic effects: updates and perspectives. *Dig Dis Sci* 2008 Jun 5 [epub].
- [34] Kekkonen RA, Sysi-Aho M, Seppanen-Laakso T, Julkunen I, Vapaatalo H, Oresic M, et al. Effect of probiotic *Lactobacillus rhamnosus* GG intervention on global serum lipidomic profiles in healthy adults. *World J Gastroenterol* 2008 May 28;14(20):3188–94.
- [35] McFarland LV, Surawicz CM, Greenberg RN, 10 other authors. A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA* 1994;271:1913–8.
- [36] Surawicz CM, McFarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, et al. The search for a better treatment for recurrent *Clostridium difficile* disease: use of high-dose vancomycin combined with *Saccharomyces boulardii*. *Clin Infect Dis* 2000;31(4):1012–7.
- [37] Pochapin M. The effect of probiotics on *Clostridium difficile* diarrhea. *Am J Gastroenterol* 2000;95(S1):S11–3.
- [38] Wullt M, Hagslatt MJ, Odénholt I. *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated diarrhoea: a double-blind, placebo-controlled trial. *Scand J Infect Dis* 2003;35:365–7.
- [39] Lawrence SJ, Korzenik JR, Mundy LM. Probiotics for recurrent *Clostridium difficile* disease. *J Med Microbiol* 2005 Sep;54(Pt 9):905–6.
- [40] Plummer S, Weaver MA, Harris JC, et al. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *Clostridium difficile* diarrhoea. *Int Microbiol* 2004;7:59–62.
- [41] Boyle RJ, et al. Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006;83:1256–64.
- [42] McCullough MJ, Clemons KV, McCusker JH, Stevens DA. Species identification and virulence attributes of *Saccharomyces boulardii* (nom. inval.). *J Clin Microbiol* 1998 Sep;36(9):2613–7.
- [43] Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkyinen S, et al. Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. *Clin Infect Dis* 1999;28:1159–60.
- [44] Boirivant M, Strober W. The mechanism of action of probiotics. *Curr Opin Gastroenterol* 2007 Nov;23(6):679–92 [PMID: 17906447].
- [45] Gu RX, Yang ZQ, Li ZH, Chen SL, Luo ZL. Probiotic properties of lactic acid bacteria isolated from stool samples of longevous people in regions of Hotan, Xinjiang and Bama, Guangxi, China. *Anaerobe* 2008 Jun 28.
- [46] McFarland LV. A review of the evidence of health claims for biotherapeutic agents. *Microb Ecol Health Dis* 2000;12:65–76.
- [47] Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* 2008 Jan;24(1):4–10.
- [48] McFarland LV. Normal flora: diversity and functions. *Microb Ecol Health Dis* 2000;12:193–207.
- [49] Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, et al. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003;171:6164–72.
- [50] Wu X, Vallance BA, Boyer L, Bergstrom KS, Walker J, Madsen K, et al. *Saccharomyces boulardii* ameliorates *Citrobacter rodentium*-induced colitis through actions on bacterial virulence factors. *Am J Physiol Gastrointest Liver Physiol* 2008 Jan;294(1):G295–306.
- [51] Castagliuolo I, LaMont JT, Nikulasson ST, Pothoulakis C. *Saccharomyces boulardii* protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. *Infect Immun* 1996;64:5225–32.
- [52] Buts JP, Dekeyser N, Stilmant C, Delem E, Smets F, Sokal E. *Saccharomyces boulardii* produces in rat small intestine a novel protein phosphatase that inhibits *Escherichia coli* endotoxin by dephosphorylation. *Pediatr Res* 2006 Jul;60(1):24–9.
- [53] Paton AW, Jennings MP, Morona R, Wang H, Focareta A, Roddam LF, et al. Recombinant probiotics for treatment and prevention of enterotoxigenic *Escherichia coli* diarrhea. *Gastroenterology* 2005 May;128(5):1219–28.
- [54] Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin a and protection against recurrent *Clostridium difficile* diarrhea. *Lancet* 2001;357:189–93.
- [55] Martin FP, Wang Y, Sprenger N, Yap IKS, Lundsted T, Lek P, et al. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* 2008;4:157 [PMID: 18197175].
- [56] Verdú EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006 Feb;55(2):182–90.
- [57] Soo I, Madsen KL, Tejpar Q, Sydora BC, Sherbaniuk R, Cincque B, et al. VSL#3 probiotic upregulates intestinal mucosal alkaline sphingomyelinase and reduces inflammation. *Can J Gastroenterol* 2008 Mar;22(3):237–42.
- [58] Barc MC, Charrin-Sarnel C, Rochet V, Bourlioux F, Sandré C, Boureau H, et al. Molecular analysis of the digestive microbiota in a gnotobiotic mouse model

- during antibiotic treatment: Influence of *Saccharomyces boulardii*. *Anaerobe* 2008 Apr 22 [Epub ahead of print].
- [59] Gorbach SL. Probiotics and gastrointestinal health. *Am J Gastroenterol* 2000 Jan;95(Suppl. 1):S2–4.
- [60] Elmer GW, McFarland LV, Surawicz CM, Danko L, Greenberg RN. Behaviour of *Saccharomyces boulardii* in recurrent *Clostridium difficile* disease patients. *Aliment Pharmacol Ther* 1999 Dec;13(12):1663–8.
- [61] Marcobal A, Underwood MA, Mills DA. Rapid determination of the bacterial composition of commercial probiotic products by terminal restriction fragment length polymorphism analysis. *J Pediatr Gastroenterol Nutr* 2008 May;46(5):608–11.
- [62] Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. *Expert Rev Anti-Infect Ther* 2006 Apr;4(2):261–75.
- [63] Jayamanne VS, Adams MR. Determination of survival, identity and stress resistance of probiotic bifidobacteria in bio-yoghurts. *Lett Appl Microbiol* 2006 Mar;42(3):189–94.
- [64] Masco L, Huys G, De Brandt E, Temmerman R, Swings J. Culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain bifidobacteria. *Int J Food Microbiol* 2005 Jul 15;102(2):221–30.
- [65] Reid G. How science will help shape future clinical applications of probiotics. *Clin Infect Dis* 2008 Feb 1;46(Suppl. 2):S62–6.